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Comparison of the Characteristics and Sex Pheromone of *Etiella behrii* (Zeller), a Newly Identified Pod Borer of Soybean in Indonesia, with *E. zinckenella* (Treit.)

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Abstract

Recently, *Etiella behrii* has been identified as a pod borer of soybean in Indonesia. This species had been presumably confused with *E. zinckenella* due to their similarity in external characters. We, therefore, compared the characteristics and sex pheromone between the two *Etiella* species. They could be accurately discriminated based on some external characters at both larval and adult stages, without examination of their genitalia. Duration of developmental stage of the insects reared on an artificial diet was 8 days longer in *E. behrii* than in *E. zinckenella* at 25°C. Premating period was 3 days longer in *E. behrii* than in *E. zinckenella.* Other biological data concerning mating and oviposition were similar between the two species. The sex pheromone of *E. behrii* was found to be a mixture of (*E*)-9-dodecenyl acetate (E9-12:Ac), (*Z*)-11-tetradecenyl acetate (Z11-14: Ac), dodecyl acetate (12:Ac), and (*E*)-11-tetradecenyl acetate (E11-14:Ac). Z11-14:Ac and E11-14:Ac are the common components in the two species. E9-12Ac and 12Ac in *E. behrii*, and 14:Ac and Z9-14:Ac in *E. zinckenella* could be key components in reproductive isolation between the two species. Moreover, the optimum dose of the mixture for capturing *E. behrii* males was much smaller than that for *E. zinckenella* males.

Discipline: Insect pest
Additional key words: external character, biology

Introduction

Two *Etiella* pod borers, *E. zinckenella* and *E. hobsoni* have been recognized as serious pests of soybean in Indonesia[8]. Their comparative characteristics and geographical distribution in Java have been reported by Naito et al.[7]. The former is distributed all over Java and the latter's distribution is restricted to West and Central Java[7]. Recently, we have observed that *E. behrii* was the predominant pod borer in soybean fields in East Java. All the individuals were identified as *E. behrii* in more than 100 larvae collected from soybean pods in Malang and Mojosari, East Java in both 1997 and 1998, although a few males of *E. zinckenella* were captured in sex pheromone traps. *E. behrii* had not been recognized as a soybean pest so far in Indonesia, although it had been recorded in Australia[1,11]. *E. behrii* and *E. zinckenella* had been presumably confused with each other in Indonesia due to their similarity in external characters[13]. It is

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Fig. 1. Comparison of external characters in larvae and adult females between *E. behrii* and *E. zinckenella*
Top: Fifth instar larvae (left: *E. behrii,* right: *E. zinckenella,* Bar=10 mm).
The first and second thoracic segments of *E. behrii* larvae are dark red in color. A pair of large black patches is located in the center in *E. behrii*, while several pairs of separated small ones are located posteriorly on the prothoracic shield in *E. zinckenella*.
Bottom: Adult females with folding wings.
A transverse band across the forewings of *E. behrii* in the anterior third part shows an anterior curvature in the resting position, while that of *E. zinckenella* is slightly curved. Two pairs of black spots consisting of scale masses can be recognized on the band in *E. behrii*.

important to discriminate between these two species for determining the predominant species in individual regions and developing an effective method of control of these pests. The present report therefore aimed at reviewing and presenting some biological data and describing the sex pheromone of the two *Etiella* species. We provided some data concerning the characters of *E. behrii* to distinguish it from *E. zinckenella*, in addition to genitalic characters[13]. A part of the present investigation had already been reported in other journals[3, 4, 12]. Biological data on *E. zinckenella* were based on the Japanese population, as we could not obtain a sufficient number of insects in Indonesia for propagation for the experiment.

External characters

1) Larva

The ground color of the larval body in both species ranges from light brown to light greenish gray. However, the first and second thoracic segments of *E. behrii* larvae are dark red in color from the 1st to 5th larval instars before the maturing stage (Fig. 1: top). This character is the most outstanding feature for differentiating *E. behrii* from *E. zinckenella* at the larval stages. At the maturing (wandering) stage, the dorsal surface of the larval body including the thoracic segments shows a faded red color in *E. behrii*, while a dark red color tinged with glossy purple in *E. zinckenella*. The pattern on the prothoracic shield in the fifth (final) instar is also very different in the two species; a pair of large black patches is located posteriorly in the center in *E. behrii*, while several pairs of small ones are located in the prothoracic shield in *E. zinckenella* (Fig. 1: top).

2) Adult

The two species are similar in external characters, but distinguishable by the presence of a transverse band across the wings in the anterior third part. The band shows an anterior curvature in the dorsal view in the rest-

Table 1. Composition of artificial diet for *Etiella* species

Ingredients	g / microplate
Soybean powder	13.8
Dried brewer's yeast	0.6
L-Cysteine hydrochloride monohydrate	0.12
myo-Inositol	0.12
Ascorbic acid	0.2
Sorbic acid	0.086
Chloramphenicol	0.024
Agar	0.38
Water	20.0

Table 2. Larval and pupal development, and fecundity of *E. behrii* and *E. zinckenella* reared on an artificial diet at 25°C under 16L:8D

Species	Larval duration (day)	Pupal duration (day)	Pupal weight (mg)	No. of eggs laid
E. behrii	27.8 ± 1.40 (34)	♀ 14.9 ± 0.81 (15)	35.7 ± 3.92 (64)	128.1 ± 67.4 (15)
		♂ 14.9 ± 0.64 (13)	39.5 ± 6.42 (52)	
E. zinckenella	19.8 ± 1.99 (36)	♀ 13.8 ± 3.44 (18)	43.7 ± 5.57 (45)	122.3 ± 62.7 (35)
		♂ 13.9 ± 2.59 (20)	46.0 ± 6.58 (50)	

Each value represents mean ± SD.
Numerals in parentheses indicate the number of insects used.

ing position and two pairs of black spots consisting of scales in *E. behrii*, while the band is slightly curved and lacks the black spots in *E. zinckenella* (Fig. 1: bottom). In both species, the male can be distinguished from the female by the enlarged base of the antennae, labial palps with a brush-like tuft of hairs[2] or the presence of a pair of brush organs between the meta- and meso- thorax[5].

Development and fecundity

E. behrii was reared successfully on an artificial diet for *E. zinckenella*[3] with slight improvement (Table 1) for more than 10 consecutive generations. Development and fecundity of the two *Etiella* species reared on the artificial diet are shown in Table 2. Mean developmental period of the larval and pupal stages was longer in *E. behrii* than in *E. zinckenella*. Mean pupal weight of *E. behrii* was lower than that of *E. zinckenella.* Male pupa was generally heavier than the female one in *E. behrii*, as in *E. zinckenella*[3,6]. Fecundity of the females was similar between the two species: a female laid more than 120 eggs in her lifetime.

Mating and oviposition

Mating and oviposition frequency of *E. behrii* and *E. zinckenella* at different ages and in the scotophase is shown in Fig. 2. The mating time tended to be later in *E. behrii* than in *E. zinckenella*. The former mated mainly from 3 to 7 h after light-off and the latter from 3 to 5 h at 25°C under a 16L:8D regime. Most of the moths copulated on the 5th to 8th days after emergence in *E. behrii*, while on the 2nd to 4th days after emergence in *E. zinckenella*. Thus, the premating period was 3 days longer in *E. behrii* than in *E. zinckenella*. The profiles of the frequency distribution of the oviposition time were almost the same in the two species: the largest number of eggs was laid during the 1st hour after light-off, with numbers decreasing thereafter. The daily oviposition frequency after mating showed a similar tendency in both species, although some *E. behrii* females lay eggs in the night when they mated.

Sex pheromone

Laboratory analysis and field experiments showed that the sex pheromone of *E. behrii* was a mixture of (*E*)-9-dodecenyl acetate (E9-12:Ac), (*Z*)-11-tetradecenyl acetate (Z11-14:Ac), dodecyl acetate (12:Ac), and (*E*)-11-tetradecenyl acetate (E11-14:Ac). Maximum attraction was obtained with a mixture of E9-12:Ac, Z11-14:Ac, 12:Ac and E11-14:Ac at the ratios of 10 : 90 : 0.7 : 6.3, respectively (Fig. 3). The sex pheromone of *E. zinckenella* has been found to consist of a mixture of 14:Ac, Z11-14:Ac, E11-14:Ac and Z9-14:Ac[9]. As Z11-14:Ac and E11-14:Ac are the common components, 14:Ac and Z9-14:Ac in *E. zinckenella* and E9-12Ac and 12Ac in *E. behrii* may be key components in the discrimination between the two species. The optimum dose of the 4-component blends for *E. behrii* was found to be 5.35 or 10.7 μg/rubber septum, for capturing more males than virgin females (Fig. 4). This dose level is very low compared with that with 309–1,030 μg/septum (Z11-14:Ac and Z9-14:Ac) for *E. zinckenella* reported in Hungary[10]. The amount of sex pheromone components extracted from *E. behrii* females was very small (<0.2 ng/female). It is considered that the reproductive isolation between the two species may be due to the difference in the quantity and quality of the sex pheromone.

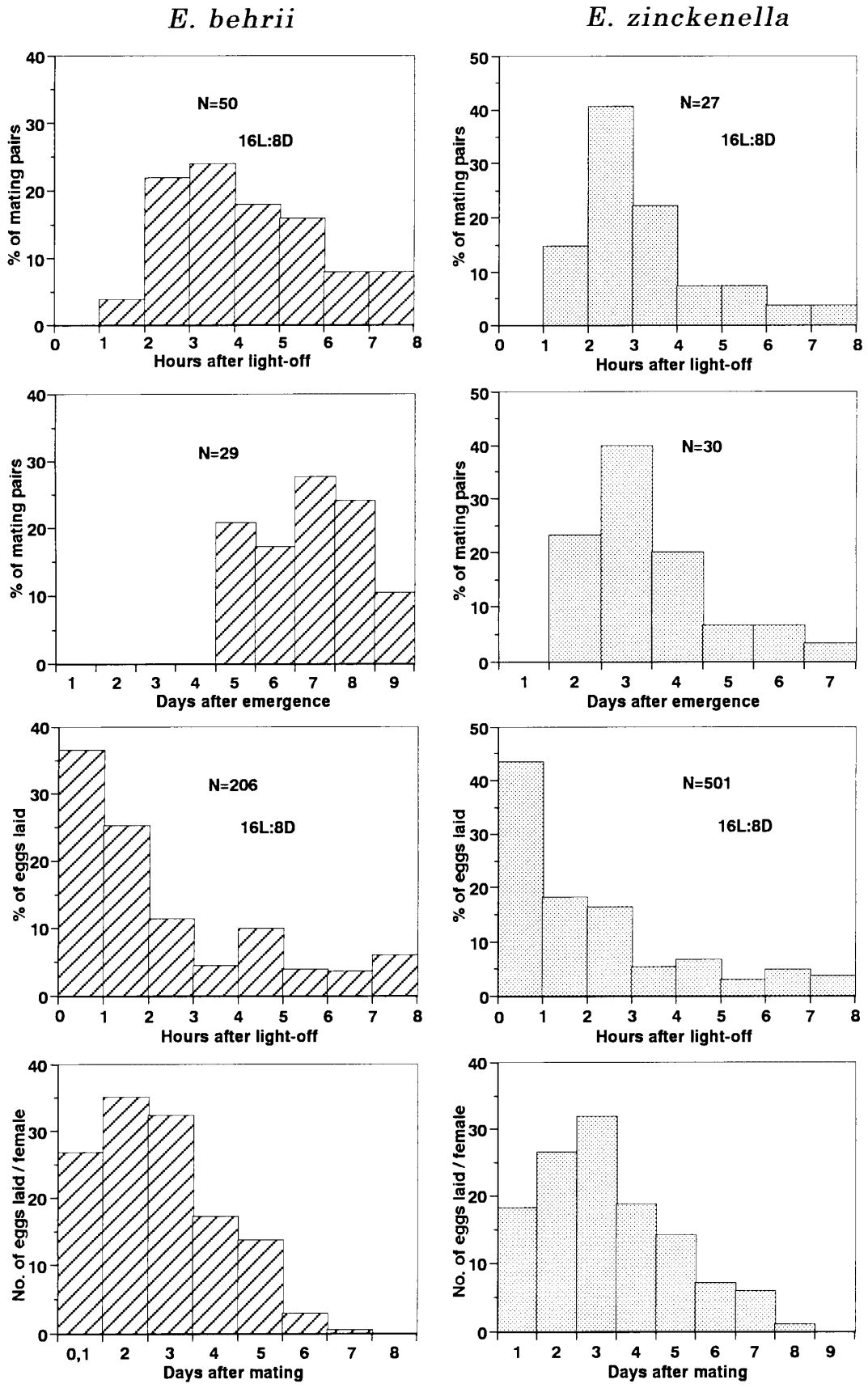

Fig. 2. Mating and oviposition frequency of *E. behrii* and *E. zinckenella* at different ages and in the scotophase

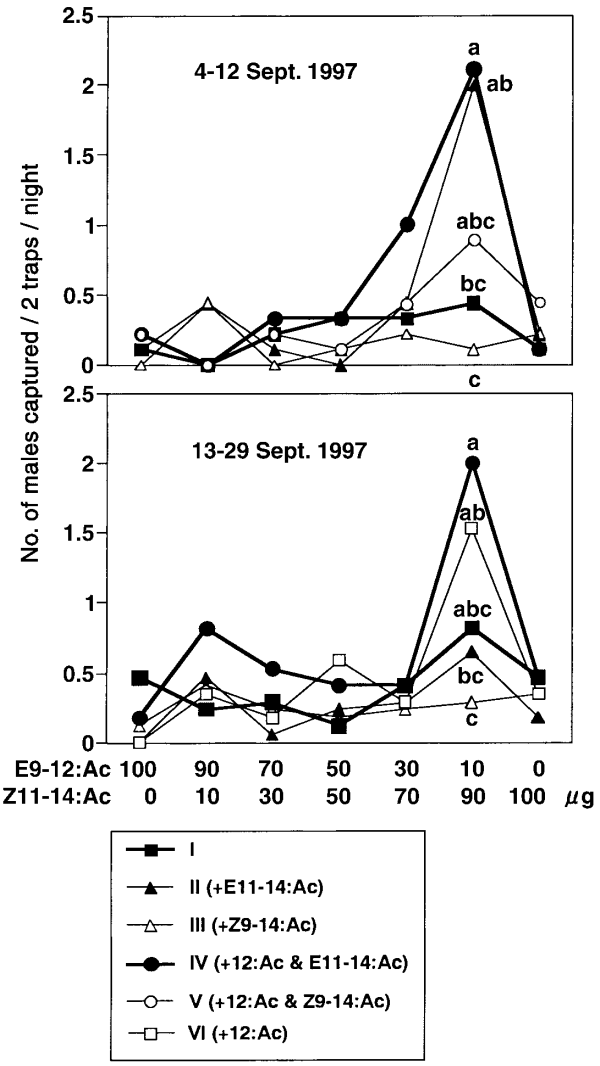

Fig. 3. Capture of *E. behrii* males with blends of E9-12:Ac and Z11-14:Ac when 12:Ac, Z9-14:Ac and/or E11-14:Ac were blended

Values with the same letter are not significantly different by Tukey's test (P=0.05).

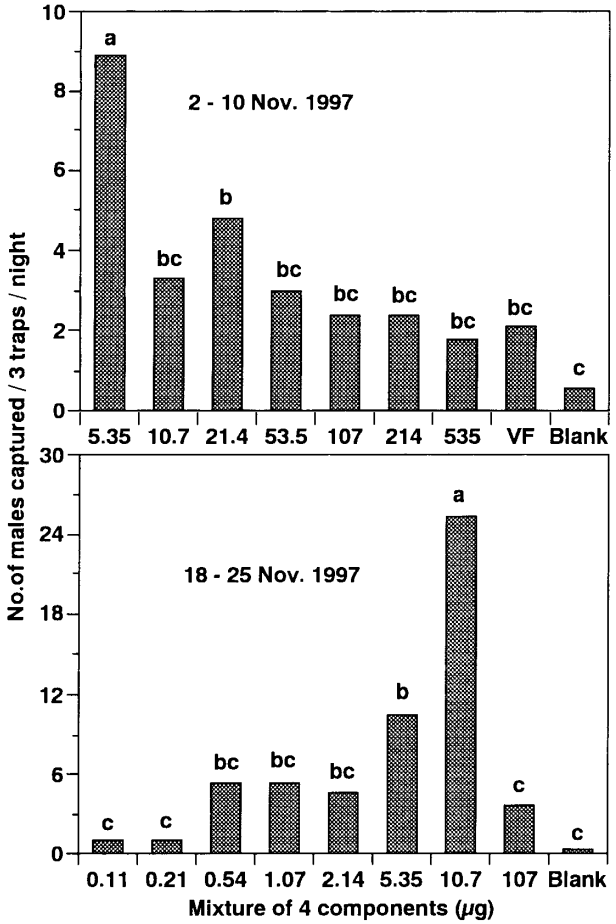

Fig. 4. Capture of *E. behrii* males with different amounts of 4-component blend

Values with the same letter are not significantly different by Tukey's test (P=0.05). E9-12:Ac, Z11-14:Ac, 12:Ac, and E11-14:Ac at the ratios of 10 : 90 : 0.7 : 6.3, respectively.

Conclusion

E. behrii and *E. zinckenella* can be reliably separated based on some external characters at both larval and adult stages, in addition to genitalic characters. Such information on external characters is useful for the discrimination of the sympatric two *Etiella* species from the practical point of view. Furthermore, the sex pheromone traps provide species-specific tools for monitoring the population of each species. Distribution of both species in the tropical and subtropical regions could be clarified by extensive surveys based on discrimination by morphological characters and monitoring by sex pheromone traps in the future. In regions where *E. behrii* is predominant, soybean damage by pod borers may be reduced by applying the mating disruption method with synthetic sex pheromone in a relatively small amount, as males of this species are very sensitive to the sex pheromone. Biological data associated with reproduction were similar between the two species, although the duration of the developmental stages and premating period was longer in *E. behrii* than in *E. zinckenella.* Further studies are required to elucidate the factors that determine the predominance between these sympatric *Etiella* species in soybean fields in East Java.

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